ANATOMY OF A GALL ON POPULUS TRICHOCARPA

Karl C. Hyde

(with plate vi)

Early in the fall of 1919 the writer collected some galls upon the twigs of *Populus trichocarpa* T. and G., caused by *Macrophoma tumefaciens* Shear,[1] in Greenough Park, Missoula, Montana. These galls were relatively large woody growths, and appeared on the trees in great numbers. Within an area of about one acre, at the north edge of the park, there were as many as twenty-five trees of various sizes bearing galls, while the trees throughout the rest of the park were practically free from knots. A close examination of the infected trees showed that nearly every twig bore from one to many galls, and that above these galls in many cases the twigs were gradually dying. The disease, although apparently occurring only in isolated localities, appeared to the writer to merit further investigation, and accordingly the problem was attacked at such an angle as to show, if possible, what effect the fungus had upon the normal twig to bring about the hypertrophy resulting in the gall.

Introduction

Populus trichocarpa is the largest tree of the genus, sometimes attaining a height of 65 m. and a diameter of 2.6 m. It is a rapid grower, and is usually found in the lowlands. It is very common throughout the Pacific coast region along the banks of streams, from southern Alaska to northern California, extending as far east as the ontinental divide in Montana. In Washington, Oregon, and eastern Montana it is the largest of the broad-leaved trees, tending to break somewhat the monotony of the vast stretches of coniferous forests of the region in its range. The tree is widely used for shade and ornament for parkings and lawns of the cities of the northwest. In Missoula, for example, there are many trees of this species within the limits of the city. It is especially adaptable for regions of light

[1] Hubert, E. E., A new *Macrophoma* on galls of *Populus trichocarpa*. Phytopath. 5:182. *figs. 3.* 1915.

rainfall because of its long roots and ease of propagation. The roots were formerly used by the Indians of California and Oregon in the manufacture of hats, baskets, mats, and other ornaments, being well adapted for this purpose because of their toughness, fineness, and length. The wood is dull brown, with nearly white sapwood, soft, light, and weak, with a specific gravity of 0.38. It shrinks moderately, warps considerably, is easily worked, but is not durable. It has a dull silky lustre, and is used extensively for cooperage, boxes, tubs, bowls, canoes, wooden legs, and paper pulp.

The only published work that treats of this gall is that of HUBERT, who deals with the characteristics of the fungus which is thought to be the cause of the gall. He originally found the galls on twigs of *Populus trichocarpa* in 1909, and at first thought they were caused by an insect, *Saperde populnea* L. In March 1910 another collection was made, and he identified the causative organism as a species of *Macrophoma*, but was unable to determine its specific identity. In November 1910 he sent samples of the galls to C. L. SHEAR, United States Bureau of Plant Industry, who described and named the fungus *Macrophoma tumefaciens*.

According to HUBERT, the fungus and galls are widely distributed throughout Montana on the twigs of *Populus trichocarpa*. The several cities mentioned as localities in which he has observed the galls show that they occur at least throughout the western part of Montana and the eastern part of Idaho.

Method

As the xylem of the normal wood is comparatively soft, no difficulty was experienced in obtaining satisfactory sections. Sections of the galls were made with a sliding microtome because they were more uniform in thickness, and good photomicrographs could be secured more readily. First the knots were cut into cubes of suitable size for clamping into the microtome. These cubes were then placed in a mixture of equal parts of glycerine, alcohol (95 per cent), and water, and heated for one hour at a temperature of 100° C. This treatment served the double purpose of softening the wood and of removing a considerable amount of air. All of the air, however, was not eliminated by this process, and it was necessary to use the airpump to remove entirely the air from the blocks. Sections 10 μ in thickness were easily obtained by these methods. In staining sections it was found that Delafield's haematoxylin in combination with safranin gave satisfactory results.

Anatomy of healthy stem

XYLEM.—The normal wood is of the diffuse, porous type (fig. 1), corresponding in this respect to other species of *Populus*. The vessels are numerous, visible to the naked eye, and are slightly larger in the spring wood than in the summer wood. They form a conspicuous ring of large pores within the earlier wood where it joins with the late wood of the previous season. In most growth rings an irregular, oblique, tangential-radial arrangement of pores can be seen, which, however, do not cross the junction of the spring wood and summer wood of the preceding season's growth. The vessels are of the bordered pitted type in the xylem of secondary growth. In the xylem that arises from the growing point, however, a great many vessels with spirally thickened walls are formed.

MEDULLARY RAY.—The rays of this species, as in all the species of *Populus* which the writer has examined, are scarcely visible with the ordinary hand lens. Only uniseriate rays have been found to occur in normal wood, the cells of which are richly supplied with simple pits (fig. 3). The ray is made up of uniform parenchyma cells elongated horizontally. Viewed radially, a ray appears as a muriform structure composed of several rows of tabular cells. The walls of the cells of the medullary ray are pitted, and the walls therefore have a lattice-like appearance when seen in radial section. The ray cells in most cases contain a considerable amount of starch.

In tangential section, the cross-sections of the rays are shown to good advantage. The uniseriate character is very evident. The rays vary from three to seventeen cells in height, and are distinctly spindle-shaped, the end cells tapering to a decided point. The cells between the end cells vary in shape from cubical to prismatic (fig. 3).

In the radial aspect one is best able to study the wood fibers that compose the bulk of the xylem. These wood fibers are slender, nonseptate, spindle-shaped, sharp-pointed cells with narrow cavities. They extend nearly parallel to one another, and diverge from their course only in weaving around the medullary rays. The wood fibers are perforated with numerous, almost circular bordered pits.

In cross-section the fibers are nearly square in outline, and have relatively thin walls. The walls are somewhat thicker in the summer wood than in the spring wood. The fibers, according to RECORD,[2] have a maximum length of 1.90 mm., a minimum length of 0.50 mm., and an average length of 1.15 mm.

BARK.—The bark as herewith considered includes all that portion of the stem outside the cambium layer, and is composed of epidermis, cortex, and phloem.

The epidermis of young twigs is smooth and colorless. Beneath this is found a layer of parenchyma, from four to seven cells thick, encircling the stem. As the stem becomes older the outer tissues disappear and are replaced by suberized tissue produced through the activity of the phellogen. In the case of normal twigs this cork tissue never becomes very thick. The fibers are thick-walled elements, with sharp pointed, unbranched ends. They are arranged in groups which are in turn arranged in bands that extend around the stem, concentric with the cambium ring.

The phloem is composed of sieve tubes, companion cells, and phloem parenchyma. In many of the cells comparatively large spherical crystals are found.

The pith rays extend into the phloem. They are uniseriate, but broaden considerably as they progress into the cortex. This broadening is due to the fact that the cells are thickened tangentially, and not to any increase in number of rows of cells.

PITH.—The central pith of the stem is composed of cylindrical cells with thin walls. The pith area is about 0.8 mm. in diameter, and varies in color from a light gray to a reddish brown. Many of the cells contain starch and crystals. The crystals are spherical in shape, and never more than one is found within a cell. The outline of the pith area in cross-section is usually distinctly five-angled, but all gradations between this and a circular outline occur.

CAMBIUM.—The cambium of this tree is similar to that of other dicotyledons. It consists of a layer of thin-walled, delicate, tabular

[2] RECORD, S. J., Economic woods of the United States. New York: Wiley and Sons. 1919.

cells. These cells have their long axes extending vertically, and are wider tangentially than radially.

Anatomy of gall

GROSS ANATOMY.—Many galls were examined and were found to vary considerably in size. The largest one measured was 3.5 cm. in diameter, and the smallest one had a diameter of 0.5 cm. They vary in shape from ovoid to globular, and usually encircle the stem. Ordinarily the galls appear singly, but it is not unusual to find them very close together or even confluent on the stem, and characteristically at the nodes. The outer surface of the gall is roughened much more than the outer portion of the normal bark, either above or below the hypertrophy. There has been such a development of suberized tissue on the periphery of the gall, due to the presence of the disease, that it becomes broken into deep, irregular, longitudinal fissures (fig. 5).

Pycnidia of *Macrophoma* are scattered irregularly over the surface of the gall. These pycnidia are flask-shaped, and are imbedded in the parenchymatous tissue of the gall. They have a pseudoparenchymatous wall and open to the exterior through an ostiolum. The pycnidia are barely visible to the naked eye, and are more abundant in the region of the fissures. Considerable sloughing of the bark from the surface occurs, especially at the time of the increased activity of the cambium region in the spring.

It is interesting to note the enormous increase of the hypertrophied part of the stem in comparison with the apparently normal stem above and below the gall. No less interesting is the macroscopic comparison of the normal wood and bark of the region below with the region through the gall (fig. 8). It shows to advantage the relative amount of increase of the tissue in question, as a result of the stimulation of the pathogene. The averages, obtained from the measurements of twenty-five galls of various sizes, are as follows:

Radius of galls	10.5 mm.
Radius of wood of galls	7.0
Thickness of bark of galls	3.5
Radius of stem immediately below gall	5.0
Radius of wood immediately below gall	4.5
Thickness of bark immediately below gall	0.5

It is apparent from these figures that the diameter of the normal stem is composed of 10 per cent bark and 90 per cent wood, while in the diseased twig 25 per cent is bark and only 75 per cent is wood. The section through the gall has increased 110 per cent, the wood 55 per cent, and the bark 600 per cent over the same tissues in the stem just below the gall.

ORIGIN.—The hypertrophy first makes its appearance on the twig in the form of a slight swelling, at or about the time of renewed cambial activity in the spring, which for the region of western Montana is about the first of May. This original swelling is brought about by the increase in number and also the increase in size of the cells of the recent phloem and xylem as secondary growth takes place. It would appear evident, therefore, that this increase in size and number of the cells is brought about by the stimulating effect produced on the cambium by the presence of the fungus. To say that this is due to enzymatic action is only a conjecture, but this is the most plausible explanation.

In the majority of knots sectioned the distortion reached to the pith, at least in some portion of the stem, which indicates that infection took place in the infancy of the twig, as xylem once formed in the region surrounding the pith ordinarily is not subsequently distorted. This, coupled with the fact that the hypertrophy is almost always formed where the twig branches, indicates that the pathogene gains entrance to the host in the region of, and during the formative period of the lateral buds.

The galls evidently arise as the result of the original infection. Frequently when a young lateral twig becomes infected its growth is stunted distad to the infection, and as a result numerous short lateral twigs are present that simulate spurs. Although these spurs may be only a few millimeters in length, they usually show several years growth, as evidenced by the number of terminal bud scars present. These spurs usually protrude from the branch at a distinct right angle, while the normal twig protrudes at an angle of approximately 30° (figs. 5–7). This increased angle is brought about mechanically, the gall forming in the axil exerting a pressure that forces the spur downward.

XYLEM.—In looking at a transverse section of diseased wood such as is shown in fig. 2, one is impressed by the enormous broadening of the rays. In this view the rays are clearly seen to be multiseriate. Occasionally the rays are seen to join together, giving rise to still wider ones. In a great many cross-sections the rays do not take a direct course through the xylem, but are often broken and their course considerably interrupted. In many cases this ray parenchyma is scattered among the wood fiber and wood parenchyma elements.

The vessels have become greatly distorted throughout (fig. 2). This is due to the flattening of the tubes in a radial direction. In most cross-sections the pores are few in number, and in sections of some galls they are entirely absent (figs. 2, 9). Considerable increase in wood parenchyma cells is seen in the diseased wood. In a great many cases the wood fibers are bent at right angles, the bend always being toward the periphery. Due to the bending, a transverse section often shows these fibers in a longitudinal view (fig. 2). This distortion is brought about largely as a result of crowding, due to the great increase in the number and size of the cells of the medullary rays.

In fig. 4 is shown a tangential section of diseased wood through a gall which illustrates to good advantage the characteristics of the medullary rays, which vary from one to several cells wide tangentially. This broadening is due to the increase in number, as well as to the increase in size of the individual cells making up the rays. That these ray cells are larger in the diseased wood than in the normal wood is apparent by comparison of fig. 3 and fig. 4, the magnification in both cases being the same. It is also evident that there is considerable increase in the size and number of the wood fiber and wood parenchyma elements. The medullary rays sometimes become so broad that their tangential diameter equals, or is even greater than their vertical diameter (fig. 4).

The average of one hundred measurements of the diameter of the medullary ray cells, in the tangential sections of normal and diseased wood of the same age, gave for the former 13.2 μ and for the latter 27.9 μ. This shows an increase of slightly over 100 per cent in these cells as a result of the diseased condition. Measurements of the diameters of the wood fibers in the same sections gave an average in the normal wood of 12 μ and in the diseased wood an average of 16 μ.

Here and there in the xylem of the diseased area uniseriate rays occur, but these are far outnumbered by the multiseriate rays. The ray tissue in the diseased xylem makes up approximately 30 per cent of the wood, while it is evident, by comparing the figures, that in the normal wood the ray tissue makes up a much smaller proportion.

Bark.—The distortion of the elements of the bark of the gall are not so pronounced as in the xylem. In the bark the principal effect is found to be a decided increase in size and number of the cells of the several tissues. As a result of the normal reaction of the host, in an attempt to overcome the injurious effects of the pathogene, the amount of suberized tissue is increased manyfold, so that now there are as many as fifty rows of cork cells. The parenchyma cells of the primary cortex are considerably larger and more than doubled in number. The average of numerous counts made of the number of rows of parenchyma cells between the cambium and the periderm in normal and diseased bark shows for the former 28, and for the latter 65. The phloem rays are multiseriate, often comprising six or seven rows of cells. These rays are sometimes bent tangentially, as seen in cross-section.

The phloem tissue is greatly increased in the diseased twig. This increase is largely due to the multiplication of the phloem parenchyma cells and the subsequent growth of the cells to a size slightly beyond the normal. Physiologically the phloem does not appear to be greatly interfered with during the younger stages of the gall. As the gall becomes larger, considerable pressure is exerted upon the sieve tubes, as is indicated by the fact that they are flattened radially.

The functional disturbance seems to be more closely connected with the xylem than with the phloem, as it is here that the distortion of elements is the greatest. This disturbance is manifested in the gradual dying of the twig above the hypertrophy (fig. 7), which, however, does not usually occur unless there are several knots upon the twig. In cases where only one knot is found on the twig there usually is no noticeable disturbance distad to the knot.

The writer believes the death of the twig to be due to the fact that sufficient water and minerals cannot get through the vessels of the xylem to the leaves beyond the galls. The supply of water and minerals is shut off because the xylem elements are so twisted and distorted that the vessels as vertical tubes and efficient water carriers have largely disappeared. The water and mineral nutrients are able to pass slowly one or two galls by diffusion, even though the vessels are greatly distorted, but when several galls are present in the path the movement is so interfered with that growth is retarded and sooner or later the tip part of the twig dies above the knot.

PITH.—No striking effects on the central pith as a result of the gall formation are noticeable, but in some larger galls the pith area is somewhat compressed, and the individual pith cells in these cases have lost their characteristic cylindrical form and have taken on an angular appearance.

CAMBIUM.—The cambium appears to be very active in the younger galls, as evidenced by the comparatively larger cells and nuclei. As the gall grows older this activity gradually grows less, until it is brought to an end by the death of the twig. In cases where death of the twig distad to the gall does not occur, the activity of the cambium eventually becomes almost negligible. The cambium ring as a whole becomes greatly distorted and interrupted in many places. The wood fibers that are bent toward the periphery are responsible for this interruption of the cambium. The cambium never entirely loses its identity (fig. 9). Many nascent cells are isolated from the cambium that give rise by continual division to isolated groups of phloem in the xylem region. These groups are more often formed between the spring wood and the summer wood of the preceding years' growth. They are usually crescent-shaped, and suggest the pith flecks often found in other woods. There are no isolated xylem groups formed in the phloem, but a great many wedgelike xylem elements extend through the cambium into this region (fig. 9).

Summary

1. The normal wood of *Populus trichocarpa* conforms closely to a typical dicotyledonous, diffuse, porous wood.

2. In western Montana and eastern Idaho a gall disease threatens to interfere with the commercial uses of this tree.

3. Only uniseriate rays are found in the normal wood of this tree, while in the diseased wood the rays are considerably broadened, often being several cells wide tangentially.

4. The average increase in the diameter of the stem, due to gall formation of several galls measured is 110 per cent, of xylem 55 per cent, and of phloem 600 per cent.

5. The xylem elements are greatly distorted, the vessels are flattened radially, and the wood fibers are often bent at right angles, due to crowding as a result of the great increase in number and size of the cells of the medullary ray.

6. In the bark the greatest effect noticeable is the increase in size and number of the parenchyma cells.

7. The phellogen is stimulated to unusual activity, and consequently the suberized tissue is considerably increased.

8. The distortion of the vascular elements, because of the interference with the transpiration stream, often results in the twigs dying above the galls.

9. The central pith is not greatly altered in the diseased stem.

10. The cambium is sometimes altered by distortion, but never completely loses its identity.

11. In addition to the evidence of constant association of *Macrophoma tumefaciens* with the lesions, the histological examination supports the idea that this fungus is the cause of the disease.

12. Infection experiments on pathogenicity are as yet lacking.

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Cornell University

EXPLANATION OF PLATE VI

All photomicrographs show magnification of 85 diameters.

Fig. 1.—Transverse section of normal wood, showing one annual ring complete.

Fig. 2.—Transverse section of diseased wood, showing distorted multiseriate medullary ray and radially flattened vessels.

Fig. 3.—Tangential section of normal wood, showing uniseriate medullary rays and normal fibers.

FIG. 4.—Tangential section of diseased wood, showing broadened medullary rays and enlarged and distorted fibers.

FIG. 5.—Twigs bearing characteristic galls; reduced $\frac{1}{3}$.

FIG. 6.—Normal twig, showing method of branching; reduced $\frac{1}{3}$.

FIG. 7.—Cluster of branches, showing characteristic habit of galls; many of these twigs were dead at tips; much reduced.

FIG. 8.—Transverse section of gall on five-year old twig; to right of this is shown transverse section of twig just below gall; reduced $\frac{1}{3}$.

FIG. 9.—Characteristic irregularity of cambium ring in diseased twig.

FIG. 10.—Normal one-year old twig and twig of same age bearing gall; reduced $\frac{1}{3}$.